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THE STRUCTURE OF ELASTEROL FROM *ECBALLIUM ELATERIUM*

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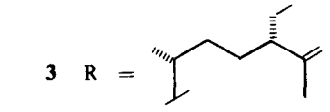
Key Word Index—*Ecballium elaterium*; Cucurbitaceae; sterols; elasterol; 24-ethylcholestanes; (24*S*)-24-ethyl-5α-cholesta-7,22,25-trien-3β-ol.

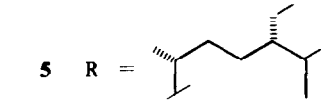
Abstract—The structure of elasterol, the major sterol of *Ecballium elaterium*, is revised to (24*S*)-24-ethyl-5α-cholesta-7,22,25-trien-3β-ol from 24-ethyl-5α-cholesta-7,16,25-trien-3β-ol.

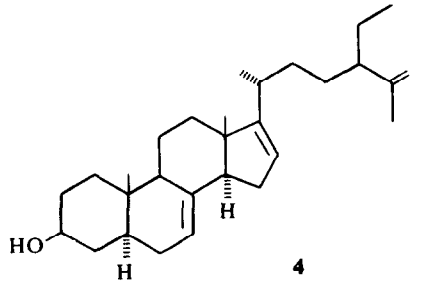
INTRODUCTION

The sterols of the Cucurbitaceae belong mainly to the 24-ethyl-5α-cholestane series [1], often with a 7-8 double bond [1–3]. For example, the main sterols of the seeds of *Cucurbita pepo*, *Cucumis melo*, *C. sativus* and *Citrullus vulgaris* are known to be 24-ethyl-5α-cholesta-7,22,25-trien-3β-ol (**1**), 24-ethyl-5α-cholesta-7,22-dien-3β-ol (**2**) and 24-ethyl-5α-cholesta-7,25-dien-3β-ol (**3**) [2, 3]. Recently these compounds have been studied by high field ^{1}H NMR and the configurations at C-24 of all of them shown to be 24*S* [4]. This is unusual in the cases of the triene (**1**) and 7,25-diene (**3**) [5], implying that two different biosynthetic pathways operate, one giving rise to **1** and **3** in the unexpected 24*S* ($24\beta_F$) series and the other to **2** (α-spinasterol) with the more usual 24*S* ($24\alpha_F$) configuration.

The sterols of *Ecballium elaterium* were investigated by Gonzalez and Panizo [6] and for the major one, which they named elasterol, the structure 24-ethyl-5α-cholesta-7,16,25-trien-3β-ol (**4**) was proposed [6]. The same workers later isolated two less abundant sterols [7] which they called dihydro- and tetrahydroelasterol giving the structures as 24-ethyl-5α-cholesta-7,16-dien-3β-ol and 24-ethyl-5α-cholest-7-en-3β-ol, respectively. We have re-examined the sterols from *Ecballium elaterium* and propose revision of these structures to those of the previously reported series. We can also assign the configuration of the 24-ethyl substituent in the side chain.

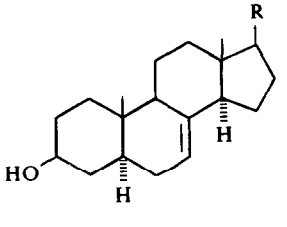


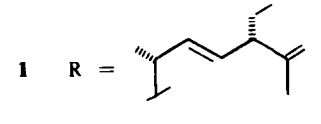


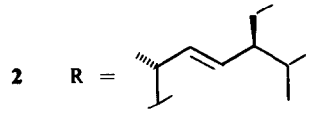


RESULTS AND DISCUSSION

The free sterol fraction of the leaves of *Ecballium elaterium* was isolated by column chromatography and the major compound, which crystallized from ethyl acetate as needles, was identical in every respect with the 'elasterol' isolated by Gonzalez and Panizo [6]. Further purification of the mother liquor after acetylation in the usual way followed by argentation preparative chromatography allowed isolation of a small amount of a second compound corresponding with the acetate of the dihydroelasterol of Gonzalez and Panizo [6]. A small amount of a more saturated compound was detected which appears to be 24-ethyl-5α-cholest-7-en-3β-ol, as indicated by Gonzalez [7].







Elasterol

The MS showed a molecular ion peak at *m/e* 410 consistent with a molecular formula of $C_{29}H_{46}O$. The IR spectrum showed absorption at 3300 cm^{-1} for an alcohol, confirmed by the easy preparation of a monoacetate on treatment with acetic anhydride in pyridine. The ^1H NMR spectrum gave signals for five vinyl hydrogens whereas Gonzalez and Panizo [6] reported resonances for only four such hydrogens. A further inconsistency concerns the reported [6, 7] resonance position of the C-29 triplet in elasteryl acetate. In view of the very large unexpected shift in position on acetylation at C-3, it can only be deduced that the reported value of δ 0.98 [6] in the spectrum of the acetate is an error. One sharp signal at δ 4.69 characteristic of a methylene group at C-26, one multiplet at 5.19 for a system such as —CH=CH— and a broad multiplet at 5.20 for one more vinyl hydrogen were observed in the spectrum. Signals for five methyl groups were observed, singlets at 0.53, 0.79 and 1.63, a doublet at 1.00 (J = 7 Hz) and a triplet centred at 0.78 (J = 7 Hz). The signal at 1.63 was assigned to a methyl group on a double bond (i.e. C-27), the doublet to the C-21 methyl group and the triplet to the C-29 methyl group of the ethyl substituent.

Thus for the side chain the only structure consistent with all these data was that of a 24-ethyl substituted normal C_{10} type with double bonds at C-22 and C-25. Confirmation of this proposal was provided by examination of the MS which had a prominent peak at *m/e* 273, corresponding with loss of such a di-unsaturated side chain. A larger peak occurred at *m/e* 271, accounted for by loss of the side chain together with two hydrogens from the nucleus, characteristic [8] of steroids with an unsaturated side chain. Assuming a cholestane-type skeleton, the structure of this compound is that of a 24-ethyl-5α-cholesta-22,25-dien-3β-ol with one more trisubstituted double bond in the nucleus. The only possible positions for such a double bond are 4, 5, 7, 9, 14 or 16 and of these 5 and 7 are most likely on biosynthetic grounds. Clarification of this point was provided by an examination of the ^1H NMR spectra of both the 5,22,25-[9] and 7,22,25-trienes [5] from which it was clear (Table 1) that the double bond could not be at C-5 and the compound was identical with 24-ethyl-5α-cholesta-7,22,25-trien-3β-ol (**1**), already isolated from several other cucurbits [3]. As further confirmation, the acetate of the material was hydrogenated with Adam's catalyst to yield a tetrahydro-derivative, identical in every respect with the acetate of dihydrochondrillasterol (**5**) [4].

With respect to the structural proof offered by Gonzalez and Panizo [6, 7] for their elasterol, a major area of discrepancy would appear to be their interpretation of the MS breakdown. There are indeed certain inconsistencies between their two publications. If their structural proposal were correct, the loss of the mono-unsaturated side chain together with the migration of two hydrogens from the nucleus would lead to a fragment at *m/e* 269, which is not observed. Instead a major peak at *m/e* 271 is present (and was interpreted by Gonzalez and Panizo [6] as loss of side chain only, without hydrogen transfer) and can only be explained by a compound with a di-unsaturated side chain. The same workers also wrongly interpreted the ^1H NMR vinyl signals leading to their incorrect structural proposal.

Sucrow *et al.* [4] have examined the high frequency ^1H NMR spectra of a range of related compounds belonging to both the 24*S* and 24*R* series and have reported slight differences in the appearance of the patterns produced by the methyl peaks, because of variations in the resonance positions of the C-26 and C-27 doublets. The coupling constants are also marginally different. By comparison of these data with those for the material isolated from *Ecballium elaterium* in the present work, it may be deduced that the configuration at C-24 is 24β_F and the structure of elasterol is (24*S*)-24-ethyl-5α-cholesta-7,22,25-trien-3β-ol (**1**). This finding confirms that of Sucrow [3] in *Cucurbita pepo* and perhaps implies that this stereochemistry is general in the Cucurbitaceae.

Dihydroelasterol

In contrast with Sucrow's work [3] on *Cucurbita pepo* seeds, we could isolate from *Ecballium elaterium* only one compound corresponding with a dihydro compound and in minute quantity which was insufficient to determine the position of the double bonds. However, the structure given by Gonzalez and Panizo for dihydroelasterol is certainly wrong and the compound can only be either 24-ethyl-5α-cholesta-7-22-dien-3β-ol (**2**) or 24-ethyl-5α-cholesta-7,25-dien-3β-ol (**3**). The ^1H NMR data given by Gonzalez and Panizo [7] are not very

Table 1. ^1H NMR data of 24-ethyl-cholestatrienes in $CDCl_3$ solution

	H-3	H-6	H-7	Me (18)	Me (19)	Me (21)	H-22, 23 or H-17	H-24	CH_2- or Me (26)	Me (27)	Me (29)	Acetate
Ecballium elaterium sterol*	3.57 (*m*)		5.20 (*m*)	0.53 (*s*)	0.79 (*s*)	1.00 (*d*, J = 7 Hz)	5.19 (*m*)	2.25-2.55 (*m*)	4.69 (*s*)	1.63 (*s*)	0.78 (*t*, J = 7 Hz)	
Ecballium elaterium steryl acetate*	4.72 (*m*)		5.22 (*m*)	0.55 (*s*)	0.82 (*s*)	1.02 (*d*, J = 7 Hz)	5.24 (*m*)	2.25-2.58 (*m*)	4.71 (*s*)	1.65 (*s*)	0.84 (*t*, J = 7 Hz)	2.01 (*s*)
Elasteryl acetate [7]	4.77 (*m*)		5.27 (*m*)	0.55 (*s*)	0.82 (*s*)	1.03 (*d*, J = 8 Hz)	5.27 (*m*)		4.77 (*s*)	1.65 (*s*)	0.98 (*t*, J = 8 Hz)	2.02 (*s*)
24-Ethyl-5α-cholesta-7,22,25-trien-3β-yl acetate [5]	4.4-4.8 (*m*)		5.1-5.3 (*m*)	0.55 (*s*)	0.82 (*s*)	1.03 (*d*, J = 7 Hz)	5.1-5.3 (*m*)	2.40 (*m*)	4.69 (*s*)	1.65 (*s*)	0.84 (*t*, J = 8 Hz)	2.01 (*s*)
24-Ethyl-cholesta-5,22,25-trien-3β-ol [9]	3.50 (*m*)	5.34 (*m*)		0.69 (*s*)	1.00 (*s*)	1.00 (*d*, J = 7 Hz)	5.21 (*m*)		4.68 (*s*)	1.64 (*s*)	0.82 (*t*, J = 7 Hz)	
Synthesized tetrahydro compound (acetate)†	4.71 (*m*)		5.18 (*m*)	0.53 (*s*)	0.82 (*s*)	0.93 (*d*, J = 6 Hz)			0.82 (*d*, J = 7 Hz)	0.82 (*d*, J = 7 Hz)	0.84 (*t*, J = 7 Hz)	2.03 (*s*)
Dihydrochondrillasteryl acetate [4]				0.53 (*s*)	0.81 (*s*)	0.93 (*d*, J = 6 Hz)			0.81 (*d*, J = 7 Hz)	0.83 (*d*, J = 7 Hz)	0.85 (*t*, J = 7 Hz)	

* 90 MHz.

† 220 MHz.

clear. However, there is no sharp signal for the two vinyl hydrogens such as would be observed for a C-26 methylene group. It is likely therefore that the dihydroelasterol of Gonzalez is 24-ethyl-5α-cholesta-7,22-dien-3β-ol (**2**). By analogy with the results of Sucrow [3] in *Cucurbita pepo*, if dihydroelasterol is a $\Delta^{7,22}$-compound, then the stereochemistry at C-24 is *S* ($24\alpha_F$), i.e. in the series opposite to that of **1** and **3**. It may be that the diene to triene ratio is variable, perhaps dependent on seasonal or environmental factors, and work is in progress to investigate this point. In any case, work already carried out [6, 7] has shown that the proportion of dihydro compounds in *Ecballium elaterium* is much less than in *Cucurbita pepo* [4].

EXPERIMENTAL

Leaves of *Ecballium elaterium* were collected from the Chelsea Physic Garden in September, 1976. After drying, the powdered material (320 g) was exhaustively extracted with petrol, bp 60–80°. Removal of the solvent under red. pres. gave a yellow-green oil (8 g) which was chromatographed over Si gel (300 g). Elution of the column with EtOAc–C_6H_6 (1:99) gave a white solid which was crystallized from EtOAc as colourless needles of 24-ethyl-5α-cholesta-7,22,25-trien-3β-ol (**1**) (128 mg); IR ν_{max}^{Nujol} cm^{-1}: 3300, 1640; MS *m/e* (rel. int.) 410 (19), 395 (10), 381 (14), 300 (19), 273 (33), 272 (22), 271 (94), 256 (11), 255 (48). Further fractions from the column were combined with the mother liquors from the isolation of **1** and acetylated in the usual way with Ac_2O in Py at room temp. overnight. After the usual work-up, PLC on Si gel G plates impregnated with $AgNO_3$ (10%) with EtOAc–cyclohexane (3:97) as developing solvent allowed isolation, in addition to a further quantity the acetate of **1**, of a compound which had a molecular ion peak in the MS at *m/e* 454, identical with the dihydroelasterol of Gonzalez and Panizo [7], probably corresponding with 24-ethyl-5α-cholesta-7,22-dien-3β-ol (**2**).

Hydrogenation of 24-ethyl-5α-cholesta-7,22,25-trien-3β-yl acetate. The acetate of **1** (15 mg) (prepared in the usual way) was dissolved in dioxan (10 ml) and hydrogenated at atmos. pres. at 45–50° for 10 hr in the presence of Adam's catalyst (10 mg). Filtration of the product through a small column of Si gel followed by PLC on Si gel plates impregnated with 10% $AgNO_3$, using EtOAc–cyclohexane (1:99) as developing solvent, allowed isolation of a white solid. This was crystallized from EtOAc–EtOH (1:1) to give colourless needles, mp 160–162°, *m/e* (rel. int.) **456 (100), 441 (21), 396 (7), 381 (11), 315 (10), 273 (14), 256 (15),** 255 (80), 229 (27), 213 (35), identical in every respect with the acetate of dihydrochondrillasterol (**5**) [4].

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